CLAIMS

1. A recombinant protein variant with the ability to induce a protective immune response to a naturally occurring allergen, wherein the protein variant:

- is a variant of a scaffold protein, said scaffold protein has a three-dimensional folding pattern that is structurally similar to that of the naturally occurring allergen,
- compared to the scaffold protein, comprises two or more primary mutations spaced by at least one non-mutated amino acid residue, each primary mutation introducing into the scaffold protein at least one amino acid residue identical or homologous to the amino acid residue or residues in corresponding position in the naturally occurring allergen, and
- has, compared to the scaffold protein, an increased affinity and/or binding capacity to IgE antibodies that are specific to the naturally occurring allergen.

2. A protein variant according to claim 1, wherein the protein variant has a reduced ability to induce histamine release compared to the naturally occurring allergen.

3. A protein variant according to claim 2 wherein the ability to induce histamine release is reduced 2 - 10.000 fold.

4. A protein variant according to claim 1, which further comprises one or more secondary mutations introducing into the scaffold protein amino acid residues, which are not present in the corresponding position in the naturally occurring allergen.

5. A protein variant according to claim 1 comprising 2-50, preferably 2-40, more preferably 3-25, more preferably 4-15 and most preferably 5-12 primary mutations.

6. A protein variant according to claim 1, wherein the scaffold protein has a level of amino acid identity with the naturally occurring allergen of between 20 and 60 %, preferably between 30 and 50 %.

7. A protein variant according to claim 1, wherein the protein variant compared to the naturally occurring allergen has a decreased binding capacity with respect to antibodies specific to the naturally occurring allergen.

8. A protein variant according to claim 1, wherein the said binding capacity is increased to at least 10 %, preferably to at least 50 %, and up to 100 % of the antibody binding capacity of the natural allergen.

9. A protein variant according to claim 1, wherein at least one of the primary mutations is a substitution.

10. A protein variant according to claim 1, wherein the introduction of at least one of the primary mutations is a deletion and/or an addition.

11. A protein variant according to claim 1, wherein the deconvoluted CD-spectra of the protein variant deviates less than 30%, preferably less than 20%, and even more preferably less than 10% compared to the deconvoluted CD-spectra of the naturally occurring allergen.

12. A protein variant according to claim 1, wherein all primary mutations are located within a surface region having an area of about 600-900 $Å^2$.

13. A protein variant according to claim 1, wherein the primary mutations comprise mutation of surface-exposed amino acids.

14. A protein variant according to claim 13, wherein the primary amino acid residues to be mutated have a solvent accessibility of above 20 %, preferably above 30 %, more preferably above 40 %, and most preferably above 50 %.

15. A protein variant according to claim 1, wherein one or more of the mutations are carried out by site-directed mutagenesis.

16. A protein variant according to claim 1, wherein one or more of the mutations are carried out by DNA shuffling.

17. A protein variant according to claim 1 obtained by gene library methods.

18. A method of producing a recombinant protein variant with the ability to induce a protective immune response to a naturally occurring allergen, comprising the steps of:

- selecting a scaffold protein, said scaffold protein having a three-dimensional folding pattern that is structurally similar to that of the naturally occurring allergen,
- introducing two or more primary mutations, that are spaced by at least one non-mutated amino acid residue, into the scaffold protein, each primary mutation introducing into the scaffold protein at least one amino acid residue identical or homologous to the corresponding amino acid residue or residues in the naturally occurring allergen, and
- the protein variant having, compared to the scaffold protein, an increased affinity and/or binding capacity to IgE antibodies that are specific to the naturally occurring protein.

19. A protein variant obtainable by the process of claim 18.

20. A protein variant according to claim 1, wherein the naturally occurring allergen is an inhalation allergen.

21. A protein variant according to claim 20, wherein the naturally occurring allergen is a pollen allergen.

22. A protein variant according to claim 21, wherein the naturally occurring allergen is a pollen allergen originating from the taxonomic order of *Fagales*, *Oleales* or *Pinales.*

23. A protein variant according to claim 22, wherein the naturally occurring allergen is *Bet v* 1.

24. A protein variant according to claim 23, wherein the scaffold protein is *Mal d* 1.

25. A protein variant according to claim 24, wherein at least two primary mutations are selected from the group consisting of: (E12V, E12I, E12M, E12L), P16A, (H40S, H40T), I43N, L44I, D47N, G65K, K70R, (E76H, E76R, E76K, Q76H), S107T, G108P, +109D, S110G, E129A, K152L, (P154S, P154T), P155S and optionally one or more secondary mutations are selected from the group consisting of: N28X, preferably N28T, K32X, preferably K32Q, E45S, E96X, +159X.

26. A protein variant (rMal d 1 (2781)) according to claim 24 comprising the sequence defined in SEQ ID NO 2, said variant comprising the following primary mutations: I43N, L44I, D47N, G65K, K70R, Q76H.

27. A protein variant (rMal d 1 (2762)) according to claim 24 comprising the sequence as defined in SEQ ID NO 3, said variant comprising the following mutations: E12V, P16A, K152L, P155S, S107T, G108P, +109D, S110G.

28. A protein variant according to claim 24 that comprise at least two primary mutations selected from the group consisting of: (E12V, E12I, E12M, E12L), (H40S, H40T), (E76H, E76R, E76K), E129A, (P154S, P154T), and optionally one or more secondary mutations selected from the group consisting of: E8X, N28X, K32X, E96X, +159X.

29. A protein variant according to claim 23 wherein the scaffold protein of Bet v 1 is Dau c 1.

30. A protein variant according to claim 29, wherein at least two primary mutations are selected from the group consisting of: (S12V, S12L, S12I, S12M), S14P, E16A, P105A, A107P, (A148S, A148T), (I151L, I151V, I151M), (N153H, N153K, N153R), (+154S, +154T), (+155D, +155E), +156A, (+157Y, +157F), (+158N, +158Q), (K39S, K39T), (K44E, K44D), (V52I, V52M, V52L), (I54K, I54R, I54H), (T64K, T64R, T64H), (T65Y, T65F, T65W), (T67K, T67R, T67H), D86E, L91G, (G92D, G92E) and optionally one or more secondary mutations are selected from the group consisting of: K32X, E42X, E59X, R69X, E95X, K122X, E8X, T10X, D25X, K32X, D46X, E59X, E95X, D108X, K122X.

31. A protein variant according to claim 29 that comprises at least two primary mutations selected from the group consisting of: (S12V, S12L, S12I, S12M), S14P, E16A, P105A, A107P, (A148S, A148T), (I151L, I151V, I151M), (N153H, N153K, N153R), (+154S, +154T), (+155D, +155E), +156A, (+157Y, +157F), (+158N, +158Q) and optionally one or more secondary mutations selected from the groups consisting of: K32X, E42X, E59X, R69X, E95X, K122X.

32. A protein variant according to claim 29 that comprises at least two primary mutations selected from the group consisting of: (K39S, K39T), (K44E, K44D), (V52I, V52M, V52L), (I54K, I54R, I54H), (T64K, T64R, T64H), (T65Y, T65F, T65W), (T67K, T67R, T67H), D86E, L91G, (G92D, G92E) and optionally at least one secondary mutation is selected from the group consisting of: E8X, T10X, D25X, K32X, D46X, E59X, E95X, D108X, K122X.

33. A protein variant according to claim 20, wherein the naturally occurring allergen originates from the taxonomic order of *Poales*.

34. A protein variant according to claim 20, wherein the naturally occurring allergen originates from the taxonomic order of *Asterales* or *Urticales*.

35. A protein variant according to claim 20, wherein the naturally occurring allergen is a house dust mite allergen.

36. A protein variant according to claim 35, wherein the naturally occurring allergen is a mite allergen originating from *Dermatophagoides*.

37. A protein variant according to claim 36, wherein the naturally occurring allergen is Der p 2.

38. A protein variant according to claim 37, wherein the scaffold protein is Eur m 1.

39. A protein variant according to claim 37, wherein the scaffold protein is Lep d 2.

40. A protein variant according to claim 39 that comprises at least two primary mutations selected from the group consisting of: D17L, D17I, D17V, D17M, S19P, Q32K, Q32R, Q32H, K33P, T35Q, T35N, N88K, N88R, N88H, T92N, T92Q, A95K, A95R, A95H and optionally one or more secondary mutations selected from the group consisting of: K6X, S22X, R30X, K76S, K81X, V114X.

41. A protein variant according to claim 39 that comprises at least two primary mutations selected from the group consisting of: D45N, D45Q, N47K, N47R, N47H, K48T, K48S, T50K, T50R, T50H, K52E, K52D, L54K, L54R, L54H, E107K, E107R, E107M, H112D, H112E, T119I, T119L, T119V, T199M and optionally one or more secondary mutations selected from the group consisting of: K6X, S22X, K29X, R30X, K76X, K81X.

42. A protein variant according to claim 37 wherein the scaffold protein is Gly d 2.

43. A protein variant according to claim 42 that comprises at least two primary mutations selected from the group consisting of: K2Q, K2N, K4D, K4E, K10N, K10Q, T14K, T14R, S22H, S22R, S22K, K39V, K39L, K39I, K39M, D45N, D45Q, T60L, T60V, T60I, T60M, Q63D, Q63E, K80V, K80L, K80I, K80M, T91S, H112D, H112E, R122I, R122V, R122L, R122M and optionally one or more secondary mutations selected from the group consisting of: K6X, preferably K6R or K6H, R30X, preferably R30K or R30H, F74X, preferably F74Y or, F74W, K81X, preferably K81R or K81H, K88X, preferably K88R or K88H, T90X, and V114X, preferably V114L, V114I or V114M.

44. A protein variant according to claim 20, wherein the naturally occurring allergen is a cockroach allergen.

45. A protein variant according to claim 19, wherein the naturally occurring allergen is an animal allergen, preferably a mammalian allergen.

46. A protein variant according to claim 45, wherein the naturally occurring allergen is an allergen originating from cat, dog or horse.

47. A protein variant according to claim 1, wherein the naturally occurring allergen is a venom allergen.

48. A protein variant according to claim 47, wherein the naturally occurring allergen originates from the taxonomic order of *Hymenoptera*.

49. A protein variant according to claim 48, wherein the naturally occurring allergen originates from the taxonomic orders of *Vespidae*, *Apidae*, or *Formicoidae*.

50. A protein variant according to claim 49, wherein the naturally occurring allergen is *Ves v* 5.

51. A protein variant according to claim 1, wherein the naturally occurring allergen is a food allergen.

52. A recombinant protein variant with the ability to modulate an immune response to a naturally occurring allergen, wherein

- the naturally occurring allergen is selected from the group consisting of plant, grass, food, and mite allergens,
- the protein variant is a variant of a scaffold protein, said scaffold protein has a three-dimensional folding pattern that is similar to that of a naturally occurring allergen, the protein variant compared to the scaffold protein comprises at least one primary mutation introducing into the scaffold protein at least one amino acid residue identical or homologous to at least one amino acid residue present in the corresponding position in the natural occurring allergen, and
- the protein variant has, compared to the scaffold protein, an increased affinity and/or binding capacity to IgE antibodies that are specific to the naturally occurring protein.

53. A recombinant protein variant with the ability to modulate an immune response to a naturally occurring allergen, wherein

- the protein variant is a variant of a scaffold protein, said scaffold protein has a three-dimensional folding pattern that is similar to that of the naturally occurring allergen,
- the scaffold protein having a level of sequence identity with the naturally occurring allergen of between 30 and 50 %,
- the protein variant compared to the scaffold protein comprises at least one primary mutation introducing into the scaffold protein at least one amino acid residue identical or homologous to at least one amino acid residue present in the corresponding position in the natural occurring allergen, and
- the protein variant has, compared to the scaffold protein, an increased affinity and/or binding capacity to IgE antibodies that are specific to the naturally occurring.

54. A recombinant protein variant with the ability to modulate an immune response to a naturally occurring allergen, wherein

- the protein variant is a variant of a scaffold protein, said scaffold protein has a three-dimensional folding pattern that is similar to that of a naturally occurring allergen,
- the deconvoluted CD-spectra of the protein variant deviates less than 30%, preferably less than 20%, and even more preferably less than 10% compared to the deconvoluted CD-spectra of the naturally occurring allergen,
- the protein variant compared to the scaffold protein comprises at least one primary mutation introducing into the scaffold protein at least one amino acid residue identical or homologous to at least one amino acid residue present in the corresponding position in the natural occurring allergen, and
- the protein variant has, compared to the scaffold protein, an increased affinity and/or binding capacity to IgE antibodies that are specific to the naturally occurring protein.

55. A pharmaceutical composition comprising protein variant according to any one of claims 1, 19 or 52-54 and a pharmaceutically acceptable carrier, excipient, or adjuvant.

56. A composition according to claim 55 comprising two or more recombinant protein variants, wherein each variant is defined by having at least one primary mutation, which is absent in at least one of the other variants.

57. A composition according to claim 56 comprising 2-12, preferably 3-10, more preferably 4-8, and most preferably 5-7 different protein variants.

58. A method of preventing or treating allergy in a patient, comprising administering to said patient the pharmaceutical composition of claim 55.

59. The pharmaceutical composition of claim 55 in the form of a vaccine against allergic reactions elicited by a naturally occurring allergen in patients suffering from allergy.

60. A method of generating an immune response in a subject comprising administering to the subject a recombinant protein variant according to any one of claims 1, 19 or 52-54.

61. A method of generating an immune response in a subject comprising administering to the subject a pharmaceutical composition according to claim 55.

62. A method of vaccination or treatment of a subject comprising administering to the subject a recombinant protein variant according to any one of claims 1, 19 or 52-54.

63. A method of vaccination or treatment of a subject comprising administering to the subject a pharmaceutical composition according to claim 55.

64. A method for the treatment, prevention or alleviation of allergic reactions in a subject comprising administering to a subject a recombinant protein variant according to any one of claims 1, 19 or 52-54.

65. A method for the treatment, prevention or alleviation of allergic reactions in a subject comprising administering to said subject a pharmaceutical composition according to claim 55.

66. A method of preparing a recombinant protein variant according to any one of claims 1, 19 or 52-54, wherein the recombinant protein variant is produced from a DNA sequence obtained by DNA shuffling (molecular breeding) of DNA encoding the scaffold protein and the naturally occurring allergen.

67. A DNA sequence encoding a recombinant protein variant according to any one of claims 1, 19 or 52-54.

68. A DNA sequence according to claim 67, wherein the derivative is obtained by site-directed mutagenesis of the DNA encoding the scaffold protein.

69. A DNA sequence according to any of claim 68, wherein the sequence is a derivative of a sequence selected from the group consisting of: SEQ ID NOs 2 and 3.

70. An expression vector comprising the DNA according to claim 67.

71. A host-cell comprising the expression vector of claim 70.

72. A method of producing a recombinant mutant protein variant comprising the step of cultivating a host T-cell according to claim 71.

73. A recombinant protein variant according to any one of claims 1, 19 or 52-54 comprising at least one T-cell epitope capable of stimulating a T-cell clone or T-cell line specific for the naturally occurring allergen.

74. A diagnostic assay for accessing relevance, safety, or outcome of therapy of a subject comprising mixing an IgE sample of said subject with a protein variant according to any one of claims 1, 19 or 52-54 and assessing the level of reactivity between the IgE in said sample and said protein variant.

75. A diagnostic assay for accessing relevance, safety, or outcome of therapy of a subject comprising mixing an IgE sample of said subject with a pharmaceutical comprisition according to claim 55 and assessing the level of reactivity between the IgE in said sample and said pharmaceutical composition.